PHENYLALANINE ENAMIDE DERIVATIVES

This invention relates to a number of phenylalanine enamide esters, to compositions containing them, to processes for their preparation, and to their use in medicine.

Over the last few years it has become increasingly clear that the physical interaction of inflammatory leukocytes with each other and other cells of the body plays an important role in regulating immune and inflammatory responses [Springer, T. A., Nature, 346, 425, (1990); Springer, T. A., Cell, 76, 301, (1994)]. Specific cell surface molecules collectively referred to as cell adhesion molecules mediate many of these interactions.

The adhesion molecules have been sub-divided into different groups on the basis of their structure. One family of adhesion molecules which is believed to play a particularly important role in regulating immune and inflammatory responses is the integrin family. This family of cell surface glycoproteins has a typical non-covalently linked heterodimer structure. At least 16 different integrin alpha chains and 8 different integrin beta chains have been identified [Newman, P. *et al*, Molecular Medicine Today, 304, (1996)]. The members of the family are typically named according to their heterodimer composition although trivial nomenclature is widespread in the field. Thus the integrin $\alpha 4\beta 1$ consists of the integrin alpha 4 chain associated with the integrin beta 1 chain, but is also widely referred to as Very Late Antigen 4 or VLA-4. Not all of the potential pairings of integrin alpha and beta chains have yet been observed in nature and the integrin family has been subdivided into a number of subgroups based on the pairings that have been recognised to date [Sonnenberg, A., Current Topics in Microbiology and Immunology, 184, 7, (1993)].

The importance of integrin function in normal physiological responses is highlighted by two human deficiency diseases in which integrin function is

defective. Thus in the disease termed Leukocyte Adhesion Deficiency (LAD) there is a defect in one of the families of integrins expressed on leukocytes [Marlin, S. D. *et al*, J. Exp. Med. 164, 855, (1986)]. Patients suffering from this disease have a reduced ability to recruit leukocytes to inflammatory sites and suffer recurrent infections, which in extreme cases may be fatal. In the case of patients suffering from the disease termed Glanzman's thrombasthenia (a defect in a member of the beta 3 integrin family) there is a defect in blood clotting (Hodivala-Dilke, K. M., J. Clin. Invest. 103, 229, (1999)].

The potential to modify integrin function in such a way as to beneficially modulate cell adhesion has been extensively investigated in animal models using specific antibodies and peptides that block various functions of these molecules [e.g. Issekutz, T. B., J. Immunol. 149, 3394, (1992); Li, Z. *et al*, Am. J. Physiol. 263, L723, (1992); Mitjans, F. *et al*, J. Cell Sci. 108, 2825, (1995); Brooks, P. C. *et al*, J. Clin. Invest. 96, 1815, (1995); Binns, R. M. *et al*, J. Immunol. 157, 4094, (1996); Hammes, H.-P. *et al*, Nature Medicine 2, 529, (1996); Srivata, S. *et al*, Cardiovascular Res. 36, 408 (1997)]. In particular an anti $\alpha_4\beta_7$-antibody has demonstrated both clinical and histologic improvement of inflammatory activity and disease in a non-human primate model of inflammatory bowel disease [Hesterberg, P.E. *et al*, Gastroenterol, 111, 1373-80 (1996)]. A number of monoclonal antibodies which block integrin function are currently being investigated for their therapeutic potential in human disease, and one, ReoPro, a chimeric antibody against the platelet integrin αIIbβ3 is in use as a potent anti-thrombotic agent for use in patients with cardiovascular complications following coronary angioplasty.

Integrins recognize both cell surface and extracellular matrix ligands, and ligand specificity is determined by the particular alpha-beta subunit combination of the molecule [Newman, P., *ibid*]. One particular integrin subgroup of interest involves the α4 chain which can pair with two different

beta chains β1 and β7 [Sonnenberg, A., *ibid*]. The α4β1 pairing occurs on many circulating leukocytes (for example lymphocytes, monocytes, eosinophils and basophils) although it is absent or only present at low levels on circulating neutrophils. α4β1 binds to an adhesion molecule (Vascular Cell Adhesion Molecule-1 also known as VCAM-1) frequently up-regulated on endothelial cells at sites of inflammation [Osborne, L., Cell, 62, 3, (1990)]. The molecule has also been shown to bind to at least three sites in the matrix molecule fibronectin [Humphries, M. J. *et al,* Ciba Foundation Symposium, 189, 177, (1995)]. Based on data obtained with monoclonal antibodies in animal models it is believed that the interaction between α4β1 and ligands on other cells and the extracellular matrix plays an important role in leukocyte migration and activation [Yednock, T. A. *et al,* Nature, 356, 63, (1992); Podolsky, D. K. *et al*, J. Clin. Invest. 92, 372, (1993); Abraham, W. M. *et al,* J. Clin. Invest. 93, 776, (1994)].

The integrin generated by the pairing of α4 and β7 has been termed LPAM-1 [Holzmann, B. and Weissman, I. L., EMBO J. 8, 1735, (1989)]. The α4β7 pairing is expressed on certain sub-populations of T and B lymphocytes and on eosinophils [Erle, D. J. *et al,* J. Immunol. 153, 517 (1994)]. Like α4β1, α4β7 binds to VCAM-1 and fibronectin. In addition, α4β7 binds to an adhesion molecule believed to be involved in the homing of leukocytes to mucosal tissue such as gastrointestinal mucosa termed MAdCAM-1 [Berlin, C. *et al*, Cell, 74, 185, (1993)]. MAdCAM-1 is preferentially expressed in the gastrointestinal track. The interaction between α4β7 and MAdCAM-1 may also be important at sites of inflammation outside of mucosal tissue [Yang, X.-D. *et al*, PNAS, 91, 12604, (1994)].

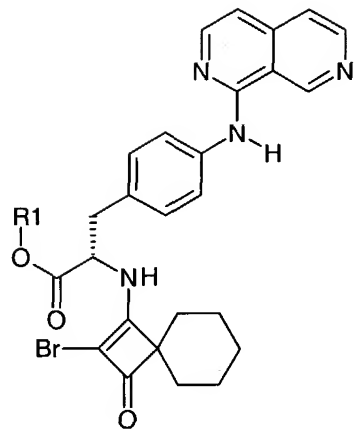
Regions of the peptide sequence recognized by α4β1 and α4β7 when they bind to their ligands have been identified. α4β1 seems to recognise LDV, IDA or REDV peptide sequences in fibronectin and a QIDSP

sequence in VCAM-1 [Humphries, M. J. *et al*, *ibid*] whilst α4β7 recognises a LDT sequence in MAdCAM-1 [Birskin, M. J. *et al,* J. Immunol. 156, 719, (1996)]. There have been several reports of inhibitors of these interactions being designed from modifications of these short peptide sequences [Cardarelli, P. M. *et al*, J. Biol. Chem., 269, 18668, (1994); Shorff, H. N.*et al,* Biorganic Med. Chem. Lett., 6, 2495, (1996); Vanderslice, P. *et al*, J. Immunol., 158, 1710, (1997)]. It has also been reported that a short peptide sequence derived from the α4β1 binding site in fibronectin can inhibit a contact hypersensitivity reaction in a trinitrochlorobenzene sensitised mouse [Ferguson, T. A., *et al*, PNAS, 88, 8072, (1991)].

Since the alpha 4 subgroup of integrins are predominantly expressed on leukocytes their inhibition can be expected to be beneficial in a number of immune or inflammatory disease states. However, because of the ubiquitous distribution and wide range of functions performed by other members of the integrin family it is important to be able to identify selective inhibitors of the alpha 4 subgroup.

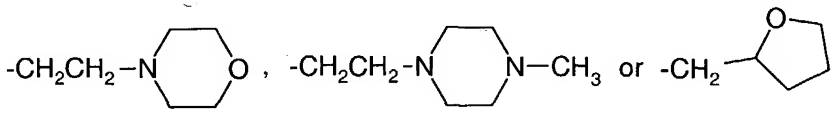
We have now found a number of esters which are potent and selective inhibitors of α4 integrins. The compounds are able to inhibit α4 integrins such as α4β1 and/or α4β7, in for example cellular assays such as those described herein, at concentrations at which they generally have no or minimal inhibitory action on α integrins of other subgroups.

Thus according to one aspect of the invention we provide a compound of formula (1):



(1)

wherein R^1 is a $-CH(CH_3)_2$, $-(CH_2)_2CH_3$, $-CH_2C(CH_3)_3$, $-CH_2CH_2OH$, $-CH_2CH_2OCH_3$, $-CH_2CH_2OCH_2CH_2OH$, $-CH_2CH_2OCH_2CH_2OCH_3$,



group;

and the salts, solvates and N-oxides thereof.

It will be appreciated that compounds of formula (1) may exist as enantiomers or diastereomers. The invention is to be understood to extend to all such enantiomers, diastereomers and mixtures thereof, including racemates. Formula (1) is intended to represent all individual isomers and mixtures thereof, unless stated or shown otherwise.

Salts of compounds of the invention include pharmaceutically acceptable salts, for example acid addition salts derived from inorganic or organic acids.

Acid addition salts include hydrochlorides, hydrobromides, hydroiodides, alkylsulphonates, e.g. methanesulphonates, ethanesulphonates, or isothionates, arylsulphonates, e.g. p-toluenesulphonates, besylates or napsylates, phosphates, sulphates, hydrogen sulphates, acetates, trifluoroacetates, propionates, citrates, maleates, fumarates, malonates, succinates, lactates, oxalates, tartrates and benzoates.

Particularly useful salts of compounds according to the invention include pharmaceutically acceptable salts, especially acid addition pharmaceutically acceptable salts.

The compounds of the invention are:
propyl (2*S*)-2-(2-bromo-3-oxospiro[3.5]non-1-en-1-ylamino)-3-[4-([2,7]naphthyridin-1-ylamino)phenyl]propanoate and the salts, solvates and N-oxides thereof;
2,2-dimethylpropyl (2*S*)-2-(2-bromo-3-oxospiro[3.5]non-1-en-1-ylamino)-3-[4-([2,7]naphthyridin-1-ylamino)phenyl]propanoate and the salts, solvates and N-oxides thereof;
2-methoxyethyl (2*S*)-2-(2-bromo-3-oxospiro[3.5]non-1-en-1-ylamino)-3-[4-([2,7]naphthyridin-1-ylamino)phenyl]propanoate and the salts, solvates and N-oxides thereof;
2-(2-hydroxyethoxy)ethyl (2*S*)-2-(2-bromo-3-oxospiro[3.5]non-1-en-1-ylamino)-3-[4-([2,7]naphthyridin-1-ylamino)phenyl]propanoate and the salts, solvates and N-oxides thereof;
2-(2-methoxyethoxy)ethyl (2*S*)-2-(2-bromo-3-oxospiro[3.5]non-1-en-1-ylamino)-3-[4-([2,7]naphthyridin-1-ylamino)phenyl]propanoate and the salts, solvates and N-oxides thereof;
2-(morpholin-4-yl)ethyl (2*S*)-2-(2-bromo-3-oxospiro[3.5]non-1-en-1-ylamino)-3-[4-([2,7]naphthyridin-1-ylamino)phenyl]propanoate and the salts, solvates and N-oxides thereof;
2-(4-methylpiperazin-1-yl)ethyl (2*S*)-2-(2-bromo-3-oxospiro[3.5]non-1-en-1-ylamino)-3-[4-([2,7]naphthyridin-1-ylamino)phenyl]propanoate and the salts, solvates and N-oxides thereof;
tetrahydrofuran-2-ylmethyl (2*S*)-2-(2-bromo-3-oxospiro[3.5]non-1-en-1-ylamino)-3-[4-([2,7]naphthyridin-1-ylamino)phenyl]propanoate and the salts, solvates and N-oxides thereof.

And more particularly:

2-hydroxyethyl (2*S*)-2-(2-bromo-3-oxospiro[3.5]non-1-en-1-ylamino)-3-[4-([2,7]naphthyridin-1-ylamino)phenyl]propanoate and the salts, solvates and N-oxides thereof;

isopropyl (2*S*)-2-(2-bromo-3-oxospiro[3.5]non-1-en-1-ylamino)-3-[4-([2,7]naphthyridin-1-ylamino)phenyl]propanoate and the salts, solvates and N-oxides thereof.

The compounds according to the invention are potent and selective inhibitors of α4 integrins. The ability of the compounds to act in this way may be simply determined by employing tests such as the cellular assays described in the Examples hereinafter.

The compounds are of use in modulating cell adhesion and in particular are of use in the prophylaxis and treatment of diseases or disorders including inflammation in which the extravasation of leukocytes plays a role and the invention extends to such a use and to the use of the compounds for the manufacture of a medicament for treating such diseases or disorders,

Diseases or disorders of this type include inflammatory arthritis such as rheumatoid arthritis, vasculitis or polydermatomyositis, multiple sclerosis, allograft rejection, diabetes, inflammatory dermatoses such as psoriasis or dermatitis, asthma and inflammatory bowel disease.

For the prophylaxis or treatment of disease the compounds according to the invention may be administered as pharmaceutical compositions, and according to a further aspect of the invention we provide a pharmaceutical composition which comprises a compound of formula (1) together with one or more pharmaceutically acceptable carriers, excipients or diluents.

Pharmaceutical compositions according to the invention may take a form suitable for oral, buccal, parenteral, nasal, topical or rectal administration, or a form suitable for administration by inhalation or insufflation.

For oral administration, the pharmaceutical compositions may take the form of, for example, tablets, lozenges or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (e.g. pregelatinised maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose); fillers (e.g. lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (e.g. magnesium stearate, talc or silica); disintegrants (e.g. potato starch or sodium glycollate); or wetting agents (e.g. sodium lauryl sulphate). The tablets may be coated by methods well known in the art. Liquid preparations for oral administration may take the form of, for example, solutions, syrups or suspensions, or they may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents, emulsifying agents, non-aqueous vehicles and preservatives. The preparations may also contain buffer salts, flavouring, colouring and sweetening agents as appropriate.

Preparations for oral administration may be suitably formulated to give controlled release of the active compound.

For buccal administration the compositions may take the form of tablets or lozenges formulated in conventional manner.

The compounds for formula (1) may be formulated for parenteral administration by injection e.g. by bolus injection or infusion. Formulations for injection may be presented in unit dosage form, e.g. in glass ampoule or multi dose containers, e.g. glass vials. The compositions for injection may take such forms as suspensions, solutions or emulsions in oily or

aqueous vehicles, and may contain formulatory agents such as suspending, stabilising, preserving and/or dispersing agents. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g. sterile pyrogen-free water, before use.

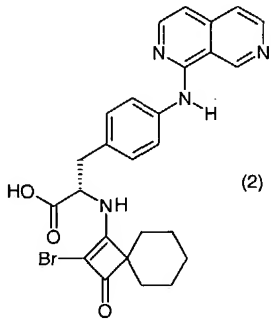
In addition to the formulations described above, the compounds of formula (1) may also be formulated as a depot preparation. Such long acting formulations may be administered by implantation or by intramuscular injection.

For nasal administration or administration by inhalation, the compounds for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation for pressurised packs or a nebuliser, with the use of suitable propellant, e.g. dichlorodifluoromethane, trichloro-fluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas or mixture of gases.

The compositions may, if desired, be presented in a pack or dispenser device which may contain one or more unit dosage forms containing the active ingredient. The pack or dispensing device may be accompanied by instructions for administration.

The quantity of a compound of the invention required for the prophylaxis or treatment of a particular condition will vary depending on the compound chosen, and the condition of the patient to be treated. In general, however, daily dosages may range from around 100ng/kg to 100mg/kg e.g. around 0.01mg/kg to 40mg/kg body weight for oral or buccal administration, from around 10ng/kg to 50mg/kg body weight for parenteral administration and around 0.05mg to around 1000mg e.g. around 0.5mg to around 1000mg for nasal administration or administration by inhalation or insufflation.

The esters of formula (1) may be prepared by the processes described in the Examples hereinafter. In general this involves esterification of an intermediate acid of formula (2):



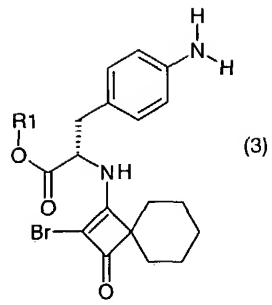
(2)

using standard methods known to those skilled in the art, such as reaction with an alcohol of formula R1OH in the presence of an acid catalyst e.g. p-toluenesulfonic acid. Alternatively a condensing agent, for example a diimide such as 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide or N,N'-dicyclohexylcarbodiimide, may be employed, advantageously in the presence of a catalyst such as a N-hydroxy compound e.g. a N-hydroxytriazole such as 1-hydroxybenzotriazole.

Intermediates of formula (2) may be prepared using methods as described in the Examples hereinafter.

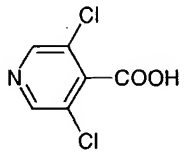
Alternatively an ester of formula (1) may undergo transesterification, preferably in the presence of an acid catalyst, to give another ester of formula (1).

Esters of formula (1) may also be prepared by coupling an amine of formula (3):



(3)

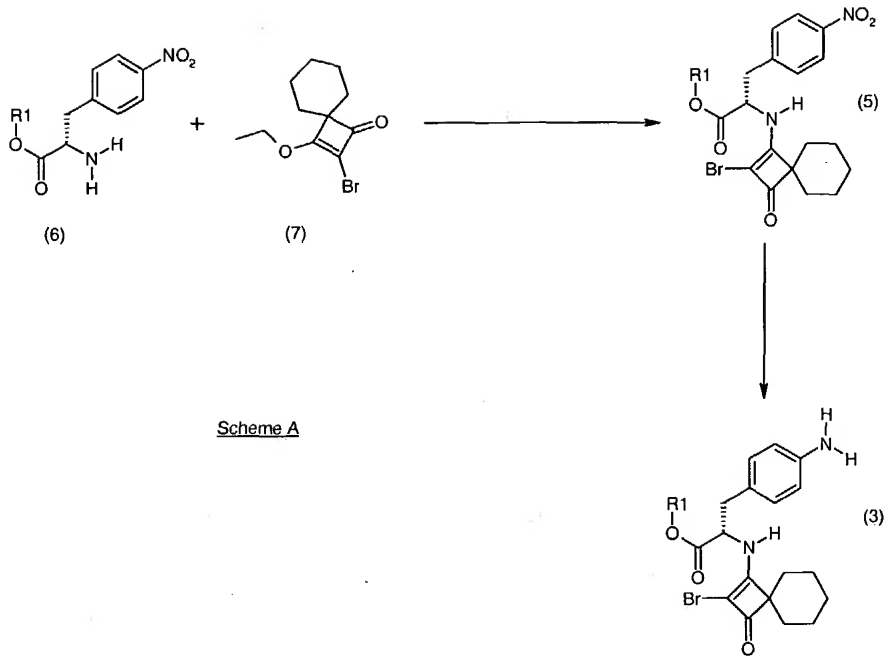
with an activated acid of formula (4):



(4)

The acid of formula (4) may be activated by conversion into an acid chloride, using standard methods known to those skilled in the art, for example, as described in the Examples hereinafter. The coupling reaction may be performed in the presence of a base, such as a hydride, e.g. sodium hydride or an amine, e.g. triethylamine or N-methylmorpholine, in a solvent such as a halogenated hydrocarbon, e.g. dichloromethane or carbon tetrachloride or a dipolar aprotic solvent such as an amide, e.g. dimethylformamide or an ether, e.g. a cyclic ether such as tetrahydrofuran, at for example ambient temperature. Alternatively, the acid of formula (4) may be coupled directly with the amine of formula (3) by the use of a condensing agent, for example a diimide such as 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide or N,N'-dicyclohexyl carbodiimide, advantageously in the presence of a catalyst such as a N-hydroxy compound e.g. a N-hydroxytriazole such as 1-hydroxybenzotriazole. Alternatively the acid may be reacted with a chloroformate, for example ethylchloroformate, prior to the desired acylation reaction.

Amines of formula (3) may be prepared using the general route as set out in Scheme A below.



Scheme A

Thus, amines of formula (3) may be prepared by reduction of a nitro compound of formula (5). Suitable conditions may involve catalytic hydrogenation using for example hydrogen in the presence of a metal catalyst, for example palladium on a support such as carbon in a solvent such as an ether, e.g. tetrahydrofuran or an alcohol e.g. methanol or ethanol. The reaction may be performed at atmospheric pressure or up to a pressure of 100 ps.i. Alternatively chemical reduction using for example a metal, e.g. tin or iron, in the presence of an acid such as hydrochloric acid may be employed.

Nitro compounds of formula (5) may be prepared by reaction of a cyclobutadiene of formula (7) with an amine of formula (6). The reaction may be performed in an inert solvent or mixture of solvents, for example a hydrocarbon such as an aromatic hydrocarbon e.g. benzene or toluene and/or a halogenated hydrocarbon such as 1,2-dichloroethane, or dichloromethane at a temperature from 0°C to the reflux temperature. Where necessary, for example when a salt of an amine of formula (6) is used, an organic base such as diisopropylethylamine can be added.

Amines of formula (6) may be prepared using standard methods known to those skilled in the art, such as esterification of commercially available 4-nitrophenylalanine.

Intermediates of formula (7) may be prepared using methods as described in International Patent Application WO 02/068393.

In addition, N-oxides of compounds of formula (1) may be prepared for example by oxidation of the corresponding nitrogen base using an oxidising agent such as hydrogen peroxide in the presence of an acid such as acetic acid, at an elevated temperature, for example around 70°C to 80°C, or alternatively by reaction with a peracid such as peracetic acid in a solvent, e.g. dichloromethane, at ambient temperature.

Salts of compounds of formula (1) may be prepared by reaction of a compound of formula (1) with an appropriate acid in a suitable solvent or mixture of solvents e.g. an organic solvent such as an ether e.g. diethylether, or an alcohol, e.g. ethanol using conventional procedures.

Where it is desired to obtain a particular enantiomer of a compound of formula (1) this may be produced from a corresponding mixture of enantiomers using any suitable conventional procedure for resolving enantiomers.

Thus for example diastereomeric derivatives, e.g. salts, may be produced by reaction of a mixture of enantiomers of formula (1) e.g. a racemate, and an appropriate chiral compound, e.g. a chiral base. The diastereomers may then be separated by any convenient means, for example by crystallisation and the desired enantiomer recovered, e.g. by treatment with an acid in the instance where the diastereomer is a salt.

In another resolution process a racemate of formula (1) may be separated using chiral High Performance Liquid Chromatography. Alternatively, if desired a particular enantiomer may be obtained by using an appropriate chiral intermediate in one of the processes described above. Alternatively, a particular enantiomer may be obtained by performing an enantiomer specific enzymatic biotransformation e.g. an ester hydrolysis using an esterase and then purifying only the enantiomerically pure hydrolysed acid from the unreacted ester antipode.

Chromatography, recrystallisation and other conventional separation procedures may also be used with intermediates or final products where it is desired to obtain a particular geometric isomer of the invention.

The following Examples illustrate the preparation of compounds of the invention. All temperatures are in °C. The following abbreviations are used:

EtOAc - ethyl acetate;	DCM - dichloromethane;
MeOH - methanol;	HOAc - acetic acid;
EtOH - ethanol;	Me - methyl;
DMSO - dimethylsulphoxide;	DMF - N,N-dimethylformamide;
THF - tetrahydrofuran,	HOBT - 1-hydroxybenzotriazole

Et_3NHCl – triethylamine hydrochloride

EDC – 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride

All NMR's were obtained either at 300MHz or 400MHz.

All Intermediates and Examples were named with the aid of Beilstein Autonom (available from MDL Information Systems GmbH, Therdor-Heuss-Allee 108D 60486, Frankfurt, Germany) or were given names that seemed consistent.

Intermediate 1 3-Cyano-4-(2-(*N.N*-dimethylamino)ethylen-1-yl)pyridine

A solution of 4-methyl-3-cyanopyridine [prepared acccording to Ref: J. Prakt. Chem. 338, 663 (1996)], (8.0g, 67.8mmol) and *N,N*-dimethylformamide diethyl acetal (11.0g, 74.8mmol) in dry DMF (50ml) was stirred at 140° under N_2 for 2 days. An additional portion of N,N,-dimethylformamide diethyl acetal (5g) was added and stirred at 140° for 4h. The volatiles were removed *in vacuo* and the obtained dark oil partitioned between EtOAc (300ml) and water (50ml). The phases were separated and the aqueous layer re-extracted with EtOAc (3x100ml). The combined organic extracts were washed with brine (30ml), dried (Na_2SO_4), treated with activated charcoal, filtered and evaporated *in vacuo* to afford essentially pure title compound as a dull orange solid (10.1g, 85%). δH ($CDCl_3$) 8.49 (1H, s), 8.25 (1h, d, J 5.9Hz), 7.29 (1H, d, J 13.2Hz), 7.09 (1H, d, J 5.9Hz), 5.25 (1H, d, J 13.2Hz) and 2.99 (6H, s); m/z (ES^+, 70V) 174 (MH^+).

Intermediate 2 **1-Hydroxy-[2,7]-naphthyridine hydrochloride salt**

HCl gas was bubbled through a stirred solution of Intermediate 1 (6.2g, 3.58mmol) in glacial acetic acid (50ml) and water (0.64ml, 3.55mmol) for 1-2min. The reaction mixture was stirred in a stoppered flask at 40° for 18h. The volatiles were removed *in vacuo* affording a dark residue, which was treated with water (3x20ml) and re-evaporated *in vacuo*. The obtained dark semi-solid was treated with 40ml warm ethanol, ice-cooled, and the undissolved solid collected by filtration affording the title compound as a green coloured solid (5.2g, 80%). δH (DMSO-d^6) 12.5 (1H, br s), 9.38 (1H, s), 8.84 (1H, d, J 7.0Hz), 8.15 (1H, d, J 7.0Hz), 7.89 (1H, br dd, J 7.0, 5.0Hz) and 6.85 (1H, d, J 7.0Hz); m/z (ES^+, 70V), 147 (MH^+).

Intermediate 3 **1-Chloro-[2,7]-naphthyridine**

Intermediate 2 (5.2g, 28.5mmol) was stirred with phosphorous oxychloride (75ml) at 110° for 24h. The volatiles were removed *in vacuo* affording a dark oil which was poured into an ice-bath cooled mixture of saturated aqueous $NaHCO_3$ (100ml containing 20g solid $NaHCO_3$) and EtOAc

(100ml). After thorough mixing the phases were separated and the aqueous layer re-extracted with EtOAc (2x75ml). The combined organic extracts were washed with brine (15ml), dried (Na_2SO_4) and evaporated *in vacuo* to afford the title compound as a yellow solid (4.0g, 85%). δH ($CDCl_3$) 9.45 (1H, s), 8.81 (1H, d, J 5.7Hz), 8.47 (1H, d, J 5.7Hz), 7.66 (1H, d, J 5.7Hz) and 7.60 (1H, d,J 5.7Hz); m/z (ES^+, 70V) 165 and 167 (MH^+).

INTERMEDIATE 4 Ethyl (2*S*)-2-amino-3-[4-([2,7]naphthyridin-1-ylamino)phenyl]propanoate

A solution of ethyl-(*S*)-3-[4-aminophenyl]-2-[*t*-butoxycarbonylamino] propanoate (638mg, 2.07mmol) and Intermediate 3 (310mg, 1.88mmol) in ethoxyethanol (2ml) was stirred at 120° for 15 min and at 100° for 1h under nitrogen. The volatiles were removed *in vacuo* and the dark residue partitioned between EtOAc (70ml) and saturated aqueous $NaHCO_3$ (10ml). The phases were separated and the aqueous layer re-extracted with EtOAc (2x30ml). The combined organic extracts were washed with brine (10ml), dried (Na_2SO_4) and evaporated *in vacuo* to afford a dark foam. Chromatography (SiO_2; 5 to 10% MeOH/DCM) afforded a mixture of ethyl-(*S*)-3-[4-([2,7]naphthyridin-1-ylamino)phenyl]-2-[(*t*-butoxycarbonyl) amino]propanoate and some of the title compound (730mg). This mixture was treated with a solution of trifluoroacetic acid (5ml) and DCM (5ml) at room temperature for 1h. The volatiles were removed *in vacuo* and the residue partitioned between EtOAc (75ml) and saturated aqueous $NaHCO_3$ (20ml). The phases were separated and the aqueous layer re-extracted with EtOAc (3x30ml). The combined organic extracts were washed with brine (10ml), dried (Na_2SO_4) and evaporated *in vacuo* to afford an orange solid. Chromatography (SiO_2; 10% MeOH/DCM) afforded the title compound as a straw-coloured solid (420mg, 60% over two steps). δH ($CDCl_3$) 10.70 (1H, s), 10.31 (1H, s), 9.44 (1H, d, J 5.6Hz), 8.94 (1H, d, J 5.6Hz), 8.55 (1H, d, J 7.3Hz), 8.54 (2H, d, J 8.5Hz), 8.46

(1H, d, J 5.6Hz), 7.94 (2H, d, J 8.5Hz), 4.84 (2H, q, J 7.1Hz), 4.35 (1H, t, J 6.6Hz), 4.10 (2H, br s), 3.64 (1H, dd, J 13.5, 6.4Hz), 3.56 (1H, dd, J 13.5, 7.0Hz) and 1.95 (3H, t, J 7.1Hz); m/z (ES+, 70V) 337 (MH^+).

Intermediate 5 Ethyl (2*S*)-3-[4-([2,7]naphthyridin-1-ylamino)phenyl]-2-(3-oxospiro[3.5]non-1-en-1-ylamino)propanoate

A solution of the ethyl ester of Intermediate 4 (565mg, 1.68mmol) and 1-keto-3-hydroxyspiro[3,5]-non-2-ene [prepared according to the method of Wasserman, H. H *et al* J. Org. Chem., 38, 1451-1455 (1973)] (280mg, 1.84mmol) in DCM (20ml) was stirred at room temperature for 24h. Concentration *in vacuo* and chromatography (SiO_2, EtOAc) of the residue gave the title compound as a pale yellow powder (1.4mmol, 73%). δH (CDCl3) 9.61 (1H, s), 8.65 (1H, d, J 5.7Hz), 8.25 (1H, d, J 5.8Hz), 7.71 (2H, d, J 8.4Hz), 7.63 (1H, d, J 8.5Hz), 7.12 (2H, d, J 8.5Hz), 7.05 (1H, d, J 5.8Hz), 5.80 (1H, m), 4.55 (1H, s), 4.29 (2H, q, J 7.2Hz), 3.13 (2H, m), 1.87–1.25 (14H, m); *m/z* (ES+, 70V) 471.1 (MH^+).

Intermediate 6 Ethyl (2*S*)-2-(2-bromo-3-oxospiro[3.5]non-1-en-1-ylamino)-3-[4-([2,7]naphthyridin-1-ylamino)phenyl]propanoate

A stirred solution of the compound of Intermediate 5 (300mg, 0.637mmol) and triethylamine (1.2eq, 100µl) in THF (10ml) at 0° was treated dropwise with a solution of bromine in DCM (2% v/v, 2.1ml, 1.2eq). After 12h the reaction was diluted with DCM (50ml) and washed successively with saturated aqueous $NaHCO_3$, dried ($MgSO_4$) filtered and concentrated *in vacuo*. The residual foam was triturated with diisopropylether and the resulting solid collected and dried *in vacuo* to give the title compound as a pale yellow powder (0.45mmol, 76%). δH (CDCl3) 9.81 (1H, s), 8.64 (1H, d, J 5.7Hz), 8.29 (1H, d, J 5.8Hz), 7.75 (2H, d, J 8.3Hz), 7.60 (1H, d, J 5.8Hz), 7.12 (2H, d, J 8.4Hz), 7.08 (1H, d, J 5.7Hz), 5.91 (1H, m), 5.03 (1H, m), 4.28 (2H, q, J 7.1Hz), 3.29 (2H, m), 1.81–1.39 (10H, m), 1.35 (3H, t, J 7.1Hz); *m/z* (ES+, 70V) 550.0 (MH^+).

Intermediat 7 (2S)-2-(2-Bromo-3-oxospiro[3.5]non-1-en-1-ylamino)-3-[4-([2,7]naphthyridin-1-ylamino)phenyl]propionic acid

The compound in Intermediate 6 (219mg, 0.40mmol) in THF (5ml) was treated in a single portion with $LiOH.H_2O$ (19mg, 0.44mmol) in H_2O (1ml) and the reaction stirred at room temperature for 2h. The reaction was then quenched by the addition of HOAc (glacial, 1ml) and the volatiles removed *in vacuo*. Water (10ml) was then added to the residual foam and stirred vigorously to effect precipitation. The precipitate was then collected by vacuum filtration and the residue washed with water (2x5ml). Drying under vacuum gave the title compound as a white powder (0.25mmol, 64%). δH (DMSO d^6, 300 K) 9.90 (1H, s), 9.56 (1H, s), 8.86 (1H,d, J 9.3Hz), 8.66 (1H, d, J 5.6Hz), 8.17 (1H, d, J 5.7Hz), 7.81 (2H, d, J 8.2Hz), 7.70 (1H, d, J 5.6Hz), 7.24 (2H, d, J 8.4Hz), 7.14 (1H, d, J 5.7Hz), 4.78 (1H, m) 3.23 (1H, dd, J 13.9, 4.1Hz), 2.99 (1H, dd, J 13.7, 10.0Hz), 1.81–1.04 (11H, m); *m/z* (ES^+, 70V) 522.0 (MH^+).

Example 1 2-Hydroxyethyl (2*S*)-2-(2-bromo-3-oxospiro[3.5]non-1-en-1-ylamino)-3-[4-([2,7]naphthyridin-1-ylamino)phenyl]propanoate

To a solution of the acid of Intermediate 7 (0.35g, 0.67mmol) in DMF (3ml) was added EDC (0.15g), HOBT (0.09g), and ethylene glycol (1ml). The mixture was stirred at room temperature overnight and then partitioned between EtOAc(20ml) and water (10ml). The organics were separated and washed with water (4 x 10ml), brine (10ml), dried (Na_2SO_4), filtered and concentrated *in vacuo* to give the crude product as yellow foam. Chromatography (SiO2, EtOAc) afforded the title compound as a yellow solid (0.25g, 66%). ^1H NMR (DMSO d6) 9.84 (1H, s), 9.53 (1H, s), 8.93 (1H, d, J = 9.3Hz), 8.66 (1H, d, J = 5.6Hz), 8.16 (1H, d, J=5.7Hz), 7.81 (2H, d, J=8.5Hz), 7.69 (1H, d, J=5.6Hz), 7.23 (2H, d, J=8.6Hz), 7.13 (1H, d, J=5.7Hz), 4.87 (2H, m), 4.19 (2H, m), 3.63 (2H, m), 3.24 (1H, dd, J=4.3,

14.0Hz), 3.02 (1H, dd, J=9.8, 13.9Hz), 1.53-1.80 (7H, m), 1.42 (1H, d, J=12.4Hz), 1.17 (2H, br). *m/z* (ESI, 70V) 567 (MH+)

Example 2 Isopropyl (2*S*)-2-(2-bromo-3-oxospiro[3.5]non-1-en-1-ylamino)-3-[4-([2,7]naphthyridin-1-ylamino)phenyl]propanoate

Solid acid from Intermediate 7 (3.6g, 6.9mmol) was added portion-wise to HCl in isopropanol, prepared beforehand by the action of acetyl chloride (10 mL) on the alcohol (100 mL) at 0-5°C. The resulting solution was left to stand at ambient temperature (20-25°C) for 16 h, checked for completion by LC, and then stripped to dryness *in vacuo*. The residue was taken up in isopropyl acetate containing triethylamine (15g) and stirred for 1h. The precipitated solid (Et_3NHCl) was filtered off and the liquors were evaporated in the presence of silica gel (10g). The pre-adsorbed product was then purified by column chromatography (SiO2, isopropyl acetate) to give the pure isopropyl ester as a yellow-green foam after removal of the solvent (3.0g, 77.4% yield). ^1H NMR ($CDCl_3$) 9.45 (1H,s), 8.68 (1H,d), 8.23 (1H, d), 7.68 (2H, d), 7.65 (1H, b,s), 7.54 (1H, d), 7.12 (2H, d), 7.05 (1H, d), 5.96 (1H, d), 5.12 (1H, septuplet), 4.95 (1H, m), 3.22 (2H, d), 1.42-1.88 (10H, m), 1.28 (6H, dd). m/z (ESI 70v) 565 (MH+).

The following cellular assays can be used to demonstrate the potency and selectivity of the compounds according to the invention. In each of these assays an IC_{50} value was determined for each test compound and represents the concentration of compound necessary to achieve 50% inhibition of cell adhesion where 100% = adhesion assessed in the absence of the test compound and 0% = absorbance in wells that did not receive cells.

$\alpha_4\beta_1$ Integrin-dependent Jurkat cell adhesion to VCAM-Ig

96 well NUNC plates were coated with $F(ab)_2$ fragment goat anti-human IgG Fcγ-specific antibody [Jackson Immuno Research 109-006-098: 100 µl at 2 µg/ml in 0.1M $NaHCO_3$, pH 8.4], overnight at 4°. The plates were washed (3x) in phosphate-buffered saline (PBS) and then blocked for 1h

in PBS/1% BSA at room temperature on a rocking platform. After washing (3x in PBS) 9 ng/ml of purified 2d VCAM-Ig diluted in PBS/1% BSA was added and the plates left for 60 minutes at room temperature on a rocking platform. The plates were washed (3x in PBS) and the assay then performed at 37° for 30 min in a total volume of 200 µl containing 2.5 x 10^5 Jurkat cells in the presence or absence of titrated test compounds.

Each plate was washed (2x) with medium and the adherent cells were fixed with 100µl methanol for 10 minutes followed by another wash. 100µl 0.25% Rose Bengal (Sigma R4507) in PBS was added for 5 minutes at room temperature and the plates washed (3x) in PBS. 100µl 50% (v/v) ethanol in PBS was added and the plates left for 60min after which the absorbance (570nm) was measured.

$\alpha_4\beta_7$ Integrin-dependent JY cell adhesion to MAdCAM-Ig

This assay was performed in the same manner as the $\alpha_4\beta_1$ assay except that MAdCAM-Ig (150ng/ml) was used in place of 2d VCAM-Ig and a sub-line of the β-lympho blastoid cell-line JY was used in place of Jurkat cells. The IC_{50} value for each test compound was determined as described in the $\alpha_4\beta_1$ integrin assay.

$\alpha_5\beta_1$ Integrin-dependent K562 cell adhesion to fibronectin

96 well tissue culture plates were coated with human plasma fibronectin (Sigma F0895) at 5µg/ml in phosphate-buffered saline (PBS) for 2 hr at 37°C. The plates were washed (3x in PBS) and then blocked for 1h in 100µl PBS/1% BSA at room temperature on a rocking platform. The blocked plates were washed (3x in PBS) and the assay then performed at 37°C in a total volume of 200µl containing 2.5x 10^5 K562 cells, phorbol-12-myristate-13-acetate at 10ng/ml, and in the presence or absence of titrated test compounds. Incubation time was 30 minutes. Each plate was fixed and stained as described in the $\alpha_4\beta_1$ assay above.

$\alpha_m\beta_2$-dependent human polymorphonuclear neutrophils adhesion to plastic

96 well tissue culture plates were coated with RPMI 1640/10% FCS for 2h at 37°C. 2 x 10^5 freshly isolated human venous polymorphonuclear neutrophils (PMN) were added to the wells in a total volume of 200µl in the presence of 10ng/ml phorbol-12-myristate-13-acetate, and in the presence or absence of test compounds, and incubated for 20min at 37°C followed by 30min at room temperature. The plates were washed in medium and 100µl 0.1% (w/v) HMB (hexadecyl trimethyl ammonium bromide, Sigma H5882) in 0.05M potassium phosphate buffer, pH 6.0 added to each well. The plates were then left on a rocker at room temperature for 60 min. Endogenous peroxidase activity was then assessed using tetramethyl benzidine (TMB) as follows: PMN lysate samples mixed with 0.22% H_2O_2 (Sigma) and 50µg/ml TMB (Boehringer Mannheim) in 0.1M sodium acetate/citrate buffer, pH 6.0 and absorbance measured at 630nm.

αIIb/β_3 -dependent human platelet aggregation

Human platelet aggregation was assessed using impedance aggregation on the Chronolog Whole Blood Lumiaggregometer. Human platelet-rich plasma (PRP) was obtained by spinning fresh human venous blood anticoagulated with 0.38% (v/v) tri-sodium citrate at 220xg for 10 min and diluted to a cell density of 6 x 10^8/ml in autologous plasma. Cuvettes contained equal volumes of PRP and filtered Tyrode's buffer (g/liter: NaCl 8.0; $MgCl_2.H_2O$ 0.427; $CaCl_2$ 0.2; KCl 0.2; D-glucose 1.0; $NaHCO_3$ 1.0; $NaHPO_4.2H_2O$ 0.065). Aggregation was monitored following addition of 2.5µM ADP (Sigma) in the presence or absence of inhibitors.

In the above assays compounds of the invention such as the compounds of the Examples generally have IC_{50} values in the $\alpha_4\beta_1$ assay of 1µM and below and in the $\alpha_4\beta_7$ assay of 5µM and below.